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# *In situ* enzymatic reclosure of opened imidazole rings of purines in DNA damaged by γ-irradiation

(γ-ray-induced base scission/DNA repair)

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**ABSTRACT** **When aqueous solutions of DNA were treated with 10–500 grays of γ-rays, the imidazole rings of some adenine and guanine residues underwent scission, resulting in the conversion of these purines to formamidopyrimidines. It was found that formamidopyrimidine-DNA glycosylase, known to remove imidazole-ring-opened 7-methylguanine from DNA, did not excise the radiation-induced non-alkylated formamidopyrimidines formed from adenine and guanine. The repair of these ring-opened purines was found to involve an enzymatic recyclizing of the opened imidazole ring that effects a restoration of the C-8 to N-9 bond. The enzyme, purine imidazole-ring cyclase reclosed the imidazole rings of 90% of ring-opened adenine or guanine, but did not close the opened imidazole ring of 7-methylguanine-derived formamidopyrimidine in DNA.**

The destruction of nucleic acid constituents by ionizing radiation (1–3) includes the cleavage of the imidazole rings of purine nucleosides and nucleotides, resulting in the conversion of adenine to 4,6-diamino-5-formamidopyrimidine (4, 5) and of guanine to 2,6-diamino-4-oxo-5-formamidopyrimidine (6). These two radiation products are generally referred to as formamidopyrimidines (FAPyr). The radiation-induced conversion of guanine to $FAPyr_{Gua}$ (7) and adenine to $FAPyr_{Ade}$ (8) in DNA has been reported. We have recently confirmed the production of $FAPyr_{Gua}$ and $FAPyr_{Ade}$ in aqueous solutions of DNA exposed to γ-irradiation (9).

The mutagenic/carcinogenic potential of FAPyr lesions has recently been highlighted by the demonstration that FAPyr residues derived from ring-opened 7-MeGua in DNA block DNA chain elongation (10). It remains to be shown whether the non-alkylated FAPyr residues have a similar effect on DNA synthesis.

When DNA containing either $FAPyr_{Ade}$ or $FAPyr_{Gua}$ was used as a substrate for formamidopyrimidine-DNA (FAPyr-DNA) glycosylase, an enzyme that excises imidazole-ring-opened 7-MeGua (MeFAPyr) (11, 12) and phosphoramide mustard-FAPyr (13) from DNA, there was no removal of either type of FAPyr residue from DNA. (Phosphoramide mustard is a metabolite of the antineoplastic agent, cyclophosphamide.) It is possible that the enzyme failed to remove these FAPyr residues because they were components of denatured DNA, which is not a good substrate for FAPyr-DNA glycosylase. We report here the discovery of a mechanism by which these radiation-induced FAPyr lesions are repaired in DNA. Rather than an excision repair of the lesions, the mechanism involves an enzymatic *in situ* reclosure of the imidazole ring to restore the C-8 to N-9 linkage previously cleaved by ionizing radiation. The imidazole ring-recyclizing activity has been named purine imidazole-ring (PurIR) cyclase. A short report of this work has been published elsewhere (14).

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## MATERIALS AND METHODS

**Materials.** 2′-Deoxy[8-$^3$H]guanosine (5 Ci/mmol; 1 Ci = 37 GBq) and 2′-deoxy[8-$^3$H]adenosine (7.8 Ci/mmol) were obtained from ICN. Deoxyribonuclease I, snake venom phosphodiesterase, spleen phosphodiesterase, and alkaline phosphatase were obtained from Sigma. Vero cells and V-79 (hamster) cells were obtained from Flow Laboratories. DNA polymerase I was from P-L Biochemicals.

**Preparation of FAPyr-DNA Substrate.** DNA was prepared (15) from a purine-requiring strain of *Bacillus subtilis* (ATCC 19162) grown in broth supplemented with either [$^3$H]deoxyadenosine or [$^3$H]deoxyguanosine at 25 μg/ml. In dose–response studies, $N_2$-saturated solutions of DNA (500 μg/ml) in 10 mM sodium phosphate, pH 7.0/1 mM EDTA were irradiated, under continuous flushing with $N_2$, with 0.1–10,000 grays (Gy, 1 Gy = 100 rads) with the University of Michigan $^{60}$Co irradiator.

The DNA then was analyzed for radiation damage. Four 20-μl aliquots of irradiated DNA were ethanol-precipitated to separate the FAPyr and purine residues remaining bound to DNA from the ethanol-soluble fraction released by γ-irradiation. The amounts of FAPyr and purine residues in these two fractions were estimated from the radioactive peaks observed upon HPLC analysis of the ethanol-soluble fraction and of the acid or nuclease hydrolysates of ethanol-precipitated DNA. The amount of FAPyr and purine residues remaining bound to DNA was also measured in the acid hydrolysates of dialyzed DNA. DNA (10 μg) was acid-hydrolyzed by 30-min incubation in 0.1 M HCl at 100°C. As acid tends to catalyze the reclosure of the opened imidazole rings of $FAPyr_{Gua}$, corrections were made for this chemical ring-reclosure when enzyme-catalyzed ring closure was determined by HPLC after acid hydrolysis. Such corrections were not necessary for $FAPyr_{Ade}$ in acid hydrolysates of DNA because no ring closure of $FAPyr_{Ade}$ occurs in acid. DNA in which $FAPyr_{Gua}$ was measured was also enzymatically hydrolyzed as described elsewhere (9). After nuclease $P_1$ (20 μg) digestion of 10 μg of DNA and adjustment of the pH of the digests to 9.0 with $NH_4OH$, 5 units of alkaline phosphatase was added; further digestion was allowed to take place for 20 hr. In some experiments the 10 μg of irradiated DNA in 20 mM Tris Cl, pH 7.5/5 mM $MgCl_2$ was sequentially digested with (*a*) 5 units of DNase I for 1 hr, (*b*) 25 units of snake venom phosphodiesterase and 25 units of spleen phosphodiesterase for 18 hr; (*c*) 5 units of alkaline phosphatase (pH 9.0) for 20 hr. Marker $FAPyr_{Ade}$ (16) and

Abbreviations: FAPyr, formamidopyrimidine (imidazole-ring-opened purine); $FAPyr_{Ade}$, 4,6-diamino-5-FAPyr (imidazole-ring-opened adenine); $FAPyr_{Gua}$, 2,6-diamino-4-oxo-5-FAPyr (imidazole-ring-opened guanine); PurIR, purine imidazole ring; PRAI, phosphoribosylaminoimidazole; $R_f$, retention time.

$FAPyr_{Gua}$ (17) were prepared according to published procedures. $FAPyr_{Ade}$ and $FAPyr_{Gua}$ from DNA digests were identified by their elution positions on HPLC relative to the elution positions of the appropriate markers; the FAPyr residues from irradiated DNA were also characterized on the basis of their ultraviolet spectra (16, 17) and their proton NMR spectra relative to those of synthetic FAPyr standards. Irradiation of DNA with a dose of 500 Gy under $N_2$ converted 45% of the guanine and 12% of the adenine to FAPyr as determined by HPLC analysis.

**Enzyme Preparation.** PurIR cyclase was prepared from lysates of 20 g of mid-log phase *Escherichia coli* B. All subsequent steps including centrifugation (12,000 × *g*) were performed at 4°C. The nucleic acids were precipitated with 1% streptomycin sulfate and removed by centrifugation. About 85% of the enzyme activity was recovered in the protein fraction that precipitated between 45% and 65% ammonium sulfate saturation. After dialysis against buffer A (0.05 M $KPO_4$, pH 7.4/1 mM EDTA), the protein was fractionated on Sephadex G-75. The active fractions were pooled and precipitated with ammonium sulfate at 65% saturation. The precipitate was collected by centrifugation, dissolved, and dialyzed against buffer B (0.025 M $KPO_4$, pH 7.4/1 mM EDTA). About 65% of the initial activity was recovered at this step. The protein was applied to a hydroxyapatite column pre-equilibrated with buffer B. Most of the applied material, including PurIR cyclase, bound to the column, while about 30% of the material was recovered in the nonbinding fraction. PurIR cyclase was eluted with buffer A, and the rest of the bound material, with buffer C (0.35 M $KPO_4$, pH 7.4/1 mM EDTA). After ammonium sulfate (65%) precipitation of the fraction eluted with buffer A and dialysis against the same buffer, 40% of the original activity was recovered. This enzyme fraction was used in the studies reported here.

**Enzyme Assay.** The assay system for meauring the enzyme-catalyzed reclosure of the imidazole rings of FAPyr residues in DNA consisted of 5 μg of irradiated [$^3$H]DNA (either [$^3$H]$FAPyr_{Ade}$-DNA or [$^3$H]$FAPyr_{Gua}$-DNA, produced by irradiation of DNA from *B. subtilis* incubated with [$^3$H]deoxyadenosine or [$^3$H]deoxyguanosine, respectively) and 4 μg of PurIR cyclase in 50 μl of 10 mM Tris Cl, pH 7.4/1 mM EDTA. Incubation was for 10 min at 37°C. To measure the amount of FAPyr with reclosed imidazole rings, the PurIR cyclase reaction was terminated by chilling the assays in an ice bath and the DNA was hydrolyzed. The method of DNA hydrolysis depended on the type of FAPyr whose ring reclosure by PurIR cyclase was being measured. When measuring ring reclosure of $FAPyr_{Ade}$, acid hydrolysis of the DNA was used, whereas ring reclosure of $FAPyr_{Gua}$ was measured both after acid and after enzymatic hydrolysis of DNA.

The hydrolysates were passed through a Sep-Pak $C_{18}$ cartridge, and the filtrates subsequently were analyzed by HPLC on a $C_{18}$ μBondapak column. The separations were carried out using a Waters Associates modular HPLC system. Isocratic elution was by means of a 3% (vol/vol) methanol/10 mM $NH_4H_2PO_4$, pH 5.1 solvent system, by which $FAPyr_{Gua}$, guanine, and deoxyguanosine, were well separated into three peaks; this was also true of $FAPyr_{Ade}$, adenine, and deoxyadenosine. From the amount of material in the FAPyr peak and that in the purine base and purine nucleoside peaks, the amount of FAPyr restored to purine structure as a result of ring reclosure by PurIR cyclase could be calculated. One unit of PurIR cyclase catalyzes the reclosure of the imidazole ring of 1 μmol of FAPyr under the reaction conditions described above.

## RESULTS

The DNA irradiated for use in these studies contained either [$^3$H]adenine or [$^3$H]guanine. To determine the threshold dose at which the scission of the imidazole ring of guanine in DNA is detectable as well as the dose at which the scission reaches a maximum, we exposed [$^3$H]guanine-DNA to various doses of γ-irradiation. By HPLC fractionation, the amount of [$^3$H]$FAPyr_{Gua}$ released from DNA by each dose of irradiation was measured in the ethanol-soluble fraction, and the amount of [$^3$H]$FAPyr_{Gua}$ remaining bound to the DNA was measured in the acid hydrolysates of ethanol-precipitated [$^3$H]guanine-DNA. The extent of radiation-induced conversion of this guanine to $FAPyr_{Gua}$ was determined on the basis of the relative amounts of radioactivity in the peaks corresponding to $FAPyr_{Gua}$, guanine, and deoxyguanosine, which were identified from their positions of elution relative to marker $FAPyr_{Gua}$. Fig. 1 shows that the amount of guanine converted to $FAPyr_{Gua}$ begins to rise after 10 Gy and reaches a plateau at 5000 Gy. The 5000-Gy dose causes extensive damage to the DNA and leaves only 23% of the $FAPyr_{Gua}$ bound to the DNA. For most of our PurIR cyclase assays, we used DNA irradiated with 500 Gy of γ-rays, because this dose left 42% of the $FAPyr_{Gua}$ bound to DNA. In some experiments, we used DNA irradiated with 10 Gy as the substrate for PurIR cyclase. This dose converts 5% of guanine to $FAPyr_{Gua}$ and releases only 10% of the $FAPyr_{Gua}$ product from the DNA.

The profile of the acid hydrolysates of γ-irradiated [$^3$H]guanine-DNA obtained by reversed-phase HPLC using isocratic elution is depicted in Fig. 2*A*. The radioactivity is eluted in peaks 1 ($R_t$ = 4.8 min) and 2 ($R_t$ = 9 min); these peaks coincide with the elution positions of marker $FAPyr_{Gua}$ and guanine, respectively. Peak 1 material has the UV absorption spectrum of $FAPyr_{Gua}$ ($\lambda_{max}$, 265 nm; $\lambda_{min}$, 230 nm). HPLC analysis of acid hydrolyzates of γ-irradiated [$^3$H]adenine-DNA in combination with marker $FAPyr_{Ade}$ and adenine revealed that 500 Gy of γ-rays converted 14% of [$^3$H]adenine ($R_t$ = 20 min) to [$^3$H]$FAPyr_{Ade}$ ($R_t$ = 4.5 min) (9).

The relative peak sizes indicate that a dose of 500 Gy converted 45% of guanine to $FAPyr_{Gua}$. This estimate was arrived at by measuring the amount of $FAPyr_{Gua}$ in γ-irradiated, undialyzed DNA. The estimates were corroborated by determining the amount of $FAPyr_{Gua}$ in ethanol-soluble material and in acid or enzymatic hydrolysates of irradiated, dialyzed DNA. The finding that 25% of $FAPyr_{Gua}$ undergoes chemical imidazole ring reclosure during acid

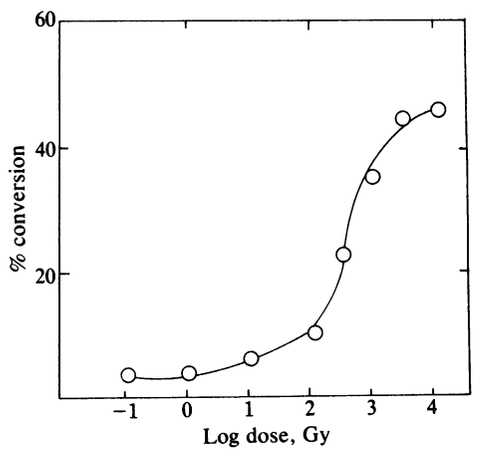


FIG. 1. Conversion of guanine in DNA to $FAPyr_{Gua}$ as a function of γ-ray dose. Two-milliliter samples containing DNA at 500 μg/ml were irradiated under $N_2$ with different doses of γ-rays. The DNA then was acid-hydrolyzed and analyzed by HPLC.

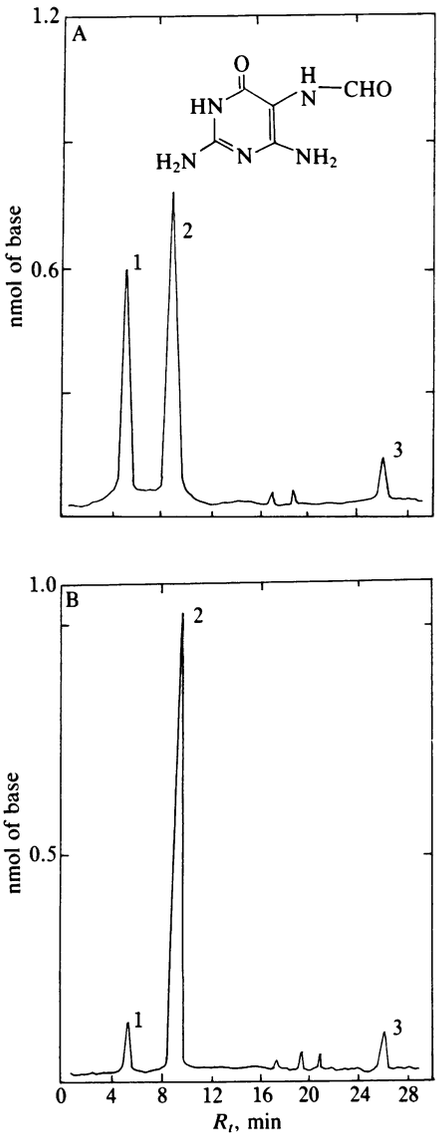


FIG. 2. HPLC separation of $FAPyr_{Gua}$ (peak 1), guanine (peak 2), and deoxyguanosine (peak 3) from nuclease $P_1$ and alkaline phosphatase hydrolysates of [$^3$H]DNA irradiated with 500 Gy. (*A*) Hydrolysate of control DNA not treated with PurIR cyclase. Insert shows the chemical structure of $FAPyr_{Gua}$. (*B*) Hydrolysate of DNA treated with PurIR cyclase and digested with 0.1 M HCl. Bases were quantitated on the basis of radioactivity.

hydrolysis necessitated correction for this acid effect. Furthermore, enzyme digestion left 15% of $FAPyr_{Gua}$ bound to DNA fragments that could not pass through Sep-Pak cartridges. After making appropriate corrections for the acid effect and the nuclease-resistant $FAPyr_{Gua}$ left in DNA, we found that the amount of $FAPyr_{Gua}$ in acid and enzyme digests of irradiated DNA was about 42% ± 4%. No imidazole ring reclosure of $FAPyr_{Ade}$ was observed after acid hydrolysis of γ-irradiated [$^3$H]adenine-DNA. An aspect of nuclease digestion of γ-irradiated DNA that is not understood is that the treatment released up to 11% of both FAPyr and purines as bases rather than as the expected nucleosides. For purposes of quantitation, we added the amounts of base and nucleoside of either FAPyr or purine to determine the quantity of each present in irradiated, dialyzed DNA.

When 500-Gy irradiated [$^3$H]guanine-DNA was treated with 4 μg of PurIR cyclase and then acid-hydrolyzed, and appropriate correction was made for 25% chemical ring-reclosure, it was determined that 90% of $FAPyr_{Gua}$ was enzymatically converted to guanine. Fig. 2*B* shows that the $FAPyr_{Gua}$ peak was reduced in proportion to the increase in the size of the guanine peak. The material in peak 2 has the UV absorption spectrum characteristic of guanine. These results show that PurIR cyclase catalyzes the reclosure of the imidazole ring previously cleaved by γ-irradiation. When we compared the effect of radiation dose on PurIR cyclase activity under the same assay conditions, we found that 1 μg of enzyme recyclized the imidazole rings of 95% of the $FAPyr_{Gua}$ residues formed (5% of total guanine) in 10-Gy irradiated DNA and of 30% of the $FAPyr_{Gua}$ residues in the same amount of 500-Gy irradiated DNA.

PurIR cyclase is active over a broad pH range (6.5–8.5) with a pH optimum of about 7.4. The imidazole ring-recyclized activity is linear over 5 min in a reaction mixture containing 5 μg of DNA and 2 μg of enzyme protein (data not shown). Comparable trends were observed in reactions in which we measured the PurIR cyclase-catalyzed conversion of $FAPyr_{Ade}$ to adenine. The enzyme has an apparent $K_m$ of 5 nM for the conversion of $FAPyr_{Ade}$ to adenine and 7 nM for $FAPyr_{Gua}$ to guanine. The apparent molecular weight of the enzyme is 32,000 (18). The enzyme retains 90% activity when stored for 1 month at 4°C. The ring recyclizing activity does not require ATP or $Mg^{2+}$.

We have examined in some detail the observed ability of PurIR cyclase to catalyze ring reclosure in the absence of an energy source. This precaution was necessitated by the demonstration by Buchanan and co-workers (19, 20) that the closure of the imidazole ring by the enzyme phosphoribosylaminoimidazole (PRAI) synthetase [5′-phosphoribosylformylglycinamide cyclo-ligase (ADP-forming); EC 6.3.3.1] during purine *de novo* synthesis requires $Mg^{2+}$ and ATP. Table 1 summarizes a series of control experiments we carried out to ascertain whether or not ATP is required for the imidazole ring closing reaction. As dehydration is associated with this ring closure, we included $NAD^+$ in some assays and found no effect. This observation agrees with the fact that $NAD^+$ is not required for the closure of the imidazole ring by PRAI synthetase during purine biosynthesis. We have prepared PRAI synthetase and characterized it by measuring its activity toward its normal substrate, 5′-phosphoribosylformylglycinamidine (19, 20), which was kindly provided by J. Stube. When PRAI synthetase was assayed for its ability to reclose the imidazole ring of either $FAPyr_{Ade}$ or $FAPyr_{Gua}$ in irradiated DNA, the enzyme did not use either one of these FAPyr residues in DNA as a substrate, even in the presence of its regular cofactors glutamine, $Mg^{2+}$ and ATP (Table 1). The results in Table 1 suggest that it is the PurIR cyclase activity that is specific for

Table 1. Effect of various cofactors on PurIR cyclase activity

| Enzyme | Cofactor(s)* | Guanine/ $FAPyr_{Gua}$ |
|---|---|---|
| None | None | 1.4 |
| PurIR cyclase | None | 11.5 |
| | $NAD^+$, $Mg^{2+}$ | 10.8 |
| | ATP | 10.7 |
| | ATP, $Mg^{2+}$ | 10.9 |
| | $NAD^+$, ATP, $Mg^{2+}$ | 9.2 |
| PRAI synthetase | ATP, $Mg^{2+}$, Gln | 1.6 |

Each assay mixture contained 5 μg of irradiated DNA and, where indicated, either 4 μg of PurIR cyclase or 10 μg of PRAI synthetase. After 30-min incubation at 37°C, the DNA was acid-hydrolyzed and analyzed by HPLC.

*Amount of cofactor added, 5 μmol.

reclosing the imidazole ring of FAPyr residues in DNA and show that this reclosure does not require ATP.

It has been shown that FAPyr-DNA glycosylase removes MeFAPyr from DNA by excision repair (11, 12). We next asked whether PurIR cyclase can also catalyze the repair of MeFAPyr-DNA by effecting imidazole-ring reclosure of MeFAPyr. Table 2 summarizes the results obtained when we compared the ability of DNA containing either $FAPyr_{Ade}$, or $FAPyr_{Gua}$, or MeFAPyr to serve as substrates for PurIR cyclase and FAPyr-DNA glycosylase. The MeFAPyr in DNA was generated by treating DNA containing 7-MeGua with 0.2 M NaOH. The solution then was neutralized and the DNA was allowed to reassociate (21) because FAPyr-DNA glycosylase prefers native DNA substrates (11, 12). The results (Table 2) show that PurIR cyclase activity is specific for nonmethylated FAPyr in DNA. Apparently, the presence of the methyl group in MeFAPyr interferes with recognition by PurIR cyclase. Note that FPyr-DNA glycosylase does not excise either $FAPyr_{Ade}$ or $FAPyr_{Gua}$ from DNA but clearly shows specificity for MeFAPyr. The PurIR cyclase preparations appear to be slightly contaminated with FAPyr-DNA glycosylase, as 4% of MeFAPyr residues were removed from DNA by the PurIR cyclase preparations used. This release of some MeFAPyr by PurIR cyclase from MeFAPyr-DNA made it necessary to determine whether FAPyr or purine residues are released from irradiated DNA by PurIR cyclase. This question was answered by precipitating the PurIR cyclase-treated DNA with chilled ethanol and analyzing the ethanol-soluble fraction by HPLC. It was found that the enzyme released an insignificant amount (3%) of either FAPyr or purine residues.

As FAPyr-DNA glycosylase prefers native MeFAPyr-DNA substrate (12), part of the explanation for the enzyme's inability to remove either $FAPyr_{Ade}$ or $FAPyr_{Gua}$ from irradiated DNA could be that the irradiation caused sufficient denaturation of the DNA to render it an unsuitable substrate for this glycosylase. When this hypothesis was tested by allowing irradiated DNA to reassociate (21) before using it as substrate in enzyme assays, it was observed that the enzyme was unable to remove non-alkylated FAPyr from the DNA, even from reassociated DNA that had been irradiated with 10-Gy of $\gamma$-rays. Although it is not possible to give a simple interpretation of these results, the 10-Gy dose is unlikely to cause as much degradation and denaturation of DNA as the 500-Gy dose. Consequently, the DNA irradiated with 500 Gy is unlikely to be recognized by FAPyr-DNA glycosylase, if this enzyme indeed does recognize non-alkylated FAPyr in DNA.

Table 2. Enzymatic reclosure of opened imidazole ring of FAPyr derived from adenine and guanine

| Irradiated DNA substrate | Enzyme: FAPyr-DNA glycosylase | Enzyme: PurIR cyclase | Radioactivity (cpm) in recyclized guanine or adenine |
|---|---|---|---|
| $FAPyr_{Gua}$-DNA | − | + | 1956 |
| $FAPyr_{Gua}$-DNA | − | − | 40 |
| $FAPyr_{Gua}$-DNA | + | − | 188 |
| MeFAPyr-DNA | − | + | 329 |
| MeFAPyr-DNA | − | − | 68 |
| MeFAPyr-DNA | + | − | 1357 |
| $FAPyr_{Ade}$-DNA | − | + | 2445 |
| $FAPyr_{Ade}$-DNA | − | − | 72 |
| $FAPyr_{Ade}$-DNA | + | − | 216 |

Each reaction mixture contained 2 $\mu$g of DNA ($\approx 6.2 \times 10^3$ cpm) and 5 $\mu$g of enzyme protein. Incubation was at 37°C for 10 min. After nuclease digestion, the hydrolysates were analyzed by HPLC. Radioactivity in reclosed adenine or guanine was computed by subtracting radioactivity found in adenine or guanine in hydrolysates of DNA not incubated with enzyme from that found in hydrolysates of enzyme-treated DNA. Data are averages from four experiments.

Table 3. Comparison of PurIR cyclase activities from different species

| Source of enzyme | Radioactivity (cpm) in recyclized adenine |
|---|---|
| — | 58 |
| *E. coli* B | 2295 |
| *E. coli* polA1 mutant | 2084 |
| *Lactobacillus casei* | 2513 |
| *Proteus mirabilis* | 2726 |
| V-79 hamster cells | 584 |
| Vero cells | 2366 |

Assay conditions were as described in the legend of Table 1, except that $FAPyr_{Ade}$-DNA was used as substrate. Five micrograms of enzyme purified through the Sephadex G-75 step was used in each assay.

Table 3 shows that PurIR cyclase is present in both bacterial and mammalian cells. PurIR cyclase activity is also present in both wild-type and polA1 *E. coli* mutants (kindly provided by Barbara Backmann of the Yale University *E. coli* Genetic Stock Center), which lack DNA polymerase I. The mammalian cells tested were the hamster cell line V-79 and the primate cell line Vero (African green monkey kidney cells). The ring-reclosing activity from Vero cells is comparable to those from three bacterial species. The enzyme activity from V-79 cells is about one-fourth as great. An examination of the levels of PurIR cyclase activity in cells sensitive to ionizing radiation may provide more insight into the biological role of this enzyme.

## DISCUSSION

We have confirmed the earlier observations that $\gamma$-irradiation of aqueous solutions of DNA induces scission of the imidazole rings of adenine (8) and guanine (7). The presence of ring-opened-purine lesions in cellular DNA is likely to have deleterious effects on the functioning of the particular region of the genome affected, since recent evidence has shown that the presence of MeFAPyr in DNA inhibits DNA synthesis by blocking chain elongation (10). The conversion of purines to FAPyr introduces a pyrimidine·pyrimidine base-pairing abnormality into the genome.

Repair of damaged purines involving a reconstitution of their normal structures is not without precedent. $O^6$-Methylguanine-DNA methyltransferase in a way performs an analogous function as it reconstitutes normal guanine structure by demethylating $O^6$-methylguanine residues in DNA (22, 23). PurIR cyclase activity has been found in bacterial and mammalian cells. The finding that FAPyr-DNA glycosylase does not excise the radiation-induced FAPyr from DNA, whereas it does excise MeFAPyr (11), phosphoramide mustard-FAPyr (13), and aflatoxin $B_1$-FAPyr (24), suggests that the excision role of this enzyme is directed at alkylated FAPyr lesions in DNA.

It was surprising to find that the ring-recyclizing reaction does not require ATP. There are two possible mechanisms underlying the ring-reclosing reaction catalyzed by PurIR cyclase: First, the electron resonance in the intact pyrimidine ring, to which the remnants of the opened imidazole ring remain bound, may become coupled to the enzyme catalysis system in a manner conducive to the restoration of the C-8 to N-9 bond. Second, the anchoring of the reacting groups to the pyrimidine ring may position them in an

orientation that facilitates ring-closure and the attendant release of a water molecule. The reason that ATP is needed in the cyclization of the imidazole-ring precursor, the linear 5'-phosphoribosyl-*N*-formylglycinamidine molecule, to form 5'-phosphoribosyl-5-aminoimidazole could be the absence of an anchoring molecule to hold the reacting groups in place. It should be noted that the last reaction in purine biosynthesis also involves an "energy-free" closure of the still-open pyrimidine ring, whose reacting groups are anchored to an intact imidazole ring (25).

The mode of repair of radiation-induced FAPyr residues in DNA described in this communication represents an expeditious process by which cells repair non-alkylated FAPyr lesions in DNA. This *in situ* repair eliminates the need for a DNA glycosylase, endonucleases, DNA polymerase, and DNA ligase (26) in the repair of DNA containing FAPyr lesions. (Mere reclosure of the imidazole ring of alkylated FAPyr would leave the biological system still having to deal with the alkyl moiety bound to the reconstituted purine.) The existence of this simple and direct repair system suggests that either natural ionizing radiation (e.g., cosmic rays) or some other FAPyr-generating mechanism may have exerted strong evolutionary pressure to which biological systems responded by producing PurIR cyclase in order to survive the impact of radiation.

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